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ORIGINAL PAPER

Role of Apolipoprotein E, Cathepsin D, and Brain-Derived Neurotrophic Factor in Parkinson's Disease: A Study from Eastern India

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disease with complex etiology. Both genetic and environmental factors play significant role. Apart from candidate genes, some modifier genes have been reported to be associated with the altered risk of PD. Previous studies have identified *Apolipoprotein E* (*APOE*), *Cathepsin D* (*CTSD*), and *Brain-Derived Neurotrophic Factor* (*BDNF*) as key players of neurodegenerative pathways with their variants associated with different neurodegenerative diseases. Hence, this study aims to identify the potential role of these modifier genes in the pathogenesis of PD among Eastern Indian PD patients. A case–control study was performed using 302 clinically diagnosed PD patients and 304 ethnically matched controls. Promoter SNPs of *APOE* (rs449647, rs405509) and *BDNF* (rs56164415), and coding SNPs of *APOE* (rs429358, rs7412 resulting in $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$ alleles), *CTSD* (rs17571), and *BDNF* (rs6265) were analyzed by PCR–RFLP and bidirectional sequencing. The effect of rs56164415 on BDNF expression was characterized by Luciferase assay. *APOEε4* allele was significantly overrepresented (p value = 0.0003) among PD patients, whereas $\varepsilon 3$ allele was predominant in the control population. The promoter haplotype (A-rs449647, G-rs405509) of *APOE* was preponderant among female PD patients posing risk. No association was found for *CTSD* polymorphism. The 'T/T' genotype of *BDNF* rs56164415 was overrepresented (p-value = 0.02) among early onset PD patients. Expression of BDNF for the 'T/T' variant was significantly lower (p-value = 0.012) than the 'C/C' variant, suggesting a possible role in PD pathogenesis. This study suggests that *APOE* and *BDNF* may serve as modifier loci among eastern Indian PD patients.

Keywords *APOE* · *CTSD* · *BDNF* · Parkinson's disease · Luciferase assay

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders characterized by the selective loss of dopaminergic neurons in substantia nigra pars compacta, resulting in reduced dopamine level in the striatum. PD is mostly sporadic, and its complex etiology may involve an interaction between multiple genetic and environmental risk factors. So far 23 PD-related loci and 19 pathogenic genes have been identified (Deng et al. 2018). Our previous studies on eastern Indian PD patients have identified a few pathogenic variants among the candidate genes, such as, *PARKIN*, *PINK1*, and *DJ-1* (Biswas et al. 2006, 2010; Sadhukhan et al. 2012). In addition to the candidate genes, many modifier genes have been reported to be associated with the pathogenesis of PD (Das et al. 2009, 2012; Sadhukhan et al. 2018; Bras et al. 2015).

Among the different pathways involved in neurodegeneration, dysfunctional mitochondrial and proteasomal degradation systems have been established for PD pathogenesis. Apolipoprotein E (APOE) plays a significant role in brain lipid transport and maintenance of synaptic plasticity and is associated with various kinds of neuropathological

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conditions. The combination of two *APOE* SNPs located in exon 4 [rs429358 (p.Cys112Arg) and rs7412 (Arg158Cys)] give rise to three isoforms, ε2 (p.Cys112/Cys112), ε3 (p.Cys112/Arg158), and ε4 (p.Arg112/Arg158), which are primarily produced by astrocytes. These three isoforms act in different ways to perform crucial functions like cholesterol transport, neuronal signaling, synaptic plasticity, and mitochondrial maintenance (Liu et al. 2013). Among these three isoforms, *APOEε4* has been found to be a potential risk factor for Alzheimer's disease (AD) (Elliott et al. 2010). The possession of one or two copies of *ε4* alleles confers a risk of approximately 5–10-fold increase in AD pathogenesis, respectively. In addition, two promoter SNPs, rs449647 (A > T) and rs405509 (T > G), also play a significant role in controlling the *APOE* gene expression (Maloney et al. 2010). Association of *APOE* with PD is extensively studied in different populations (Li et al. 2018), but there is no report from India except a study from North India (Singh et al. 2014). On the other hand, Cathepsin D (CTSD), a lysosomal/endosomal aspartic protease, has been reported to be involved in the degradation of cellular proteins including APOE, α-synuclein, which are involved in the pathogenesis of neurodegenerative diseases. A functional polymorphism, c. 224 C/T (p.Ala58Val) at exon 2 of *CTSD,* has been found to be associated with increased risk for AD (Davidson et al. 2006). Association study revealed that the combination of *APOE* (*ε4* allele) and *CTSD* (T allele) increase the risk of AD by about 19-folds (Zhou et al. 2006). AD and PD have some overlapping clinical and pathological features. Therefore, it is important to test the associations of *APOE* and *CTSD* with PD.

Brain-derived neurotrophic factor (BDNF), which is abundantly expressed in the adult mammalian brain, is a small dimeric protein, structurally related to nerve growth factor. Reduced BDNF expression has been reported in the substantia nigra of PD patients (Parain et al. 1999). BDNF plays a major role in brain development at different phases. It helps in neurogenesis, neuronal differentiation, neuronal polarization and guidance, formation and maturation of synapses, and adult synaptic plasticity suggesting that a lack of BDNF expression may be involved in PD pathogenesis. Among several polymorphisms, rs6265 (G196A) and rs56164415 (C270T) of *BDNF* gene have been extensively studied in PD patients from different populations (Dai et al. 2013), but there is no report from India. While rs6265, V66-M substitution results in reduced cell surface expression of BDNF, rs56164415 located in the seventh noncoding *BDNF* exon showing promoter-like activity can regulate the expression of BDNF (Egan et al. 2003; Pruunsild et al. 2007).

PD is a complex disease where not only the mutations in the candidate genes play an important role in PD pathogenesis, but also different modifier genes and environmental factors may increase the susceptibility of the disease. Therefore, the objective of this study is to determine the associations of *APOE*, *CTSD*, and *BDNF* in PD pathogenesis among Eastern Indian patients, which have not been studied earlier in this population group.

Methods

Study Subjects

A total of 302 PD cases whose age at onset range from 6 to 77 years (mean age at onset being 49.5 ± 12.5 years) were recruited from Bangur Institute of Neurosciences, Kolkata, India. All the patients underwent detailed neurological examination by the clinicians, where the presence of at least two cardinal features (tremor, rigidity, bradykinesia and postural instability) were the criteria for inclusion of the patients. A total of 304 healthy control (mean age being 47.9 ± 8.5 years) samples were collected from the general population, who had neither family history nor personal incidence of any neurological diseases. The healthy control samples were collected from several blood donation camps in collaboration with Institute of Blood Transfusion Medicine and Immunohaematology, Kolkata, India and Calcutta Medical College and Hospital, Kolkata, India. The study protocol adhered to the tenets of the Declaration of Helsinki and the Institutional Review Board on research using human subject approved the study after proper review as per regulation of the Indian Council of Medical Research.

APOE, *CTSD*, and *BDNF* Polymorphisms

A total of seven polymorphisms, four in *APOE*, rs449647 (A > T), rs405509 (T > G), rs429358 (T > C), and rs7412 (C > T); one in *CTSD*, rs17571 (C > T); and two in *BDNF*, rs56164415 (C > T) and rs6265 (G > A), were selected for this study. Approximately 10 ml of peripheral blood sample was collected in anticoagulant (EDTA)-containing tube with informed consent from the patients and control subjects. Genomic DNA was prepared using conventional salting-out method followed by isopropanol precipitation. DNA precipitate was dissolved in TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) and stored at 4 °C. Genotyping of the mentioned SNPs was done by PCR–RFLP and Sanger sequencing methods. All the PCR reactions were carried out using 80 ng of total genomic DNA with specific primers according to the protocol described earlier (Sadhukhan et al. 2012). The sequence of the PCR primers will be provided on request. All the PCR products were detected on a 6% polyacrylamide gel with ethidium-bromide staining. The PCR products for five SNPs from *APOE* (rs429358, rs7412), *BDNF* (rs6265, rs56164415), and *CTSD* (rs17571) were subjected

to restriction digestion with appropriate enzymes from NEB (New England Biolabs Inc. Beverly, MA) at optimum temperature (Table S1). The digested products were analyzed on a 7% polyacrylamide gel. About 10% of the samples were randomly selected and their genotypes were reconfirmed by bidirectional Sanger sequencing (ABI3130XL; Applied Biosystems, Foster City, CA). Promoter SNPs (rs449647, rs405509) of *APOE* were analyzed by Sanger sequencing.

Generation of *BDNF* C270T Variant Clones

BDNF (C270T) variants were PCR amplified from individuals with homozygous genotypes, gel purified and then simultaneously digested pGL3 basic vector using Nhe I/Hind III restriction enzymes (New England Biolabs, Beverley, MA). The digested insert and pGL3 Basic vector were ligated with *T4 DNA ligase* (Thermo Fisher Scientific, Waltham, MA, USA) and transformed into *Escherichia coli*, DH5-α. Plasmids were isolated using QIAGEN plasmid midi kit (Qiagen, Hilden, Germany), and clones were confirmed by double digestion and Sanger sequencing. Out of those that had the insert in the correct orientation, were selected for further studies.

Mammalian Cell Culture & Transfection

Mammalian cell-line SH-SY5Y was grown in high glucose DMEM (GIBCO BRL), supplemented with 10% FBS, non-essential amino acids (Thermo Fisher Scientific, Rockford, IL), L-GlutaMAX, 1% penicillin/streptomycin, and sodium pyruvate in a 37 °C incubator with 5% CO_2 under normal humidity. Cells were transiently cotransfected with 2 µg of BDNF plasmids and 2 µg of *Renilla* vector mixed with TurboFect (ThermoFisher, Rockford, IL) according to manufacturer's protocol.

Luciferase Assay

To determine the activity of *BDNF* promoter variants, Dual Luciferase assay was performed. The cells were washed with phosphate buffer saline (PBS) 48 h after transfection and subsequently lysed with luciferase cell lysis buffer supplied with the Dual Luciferase Reporter Assay kit (Promega, Madison, WI). The cell lysates were centrifuged at 13,000 rpm for 1 min at 4 °C, and 10 µl of supernatants was mixed with 25 µl of luciferase assay reagent-I; Firefly (FF) luminescence was measured as relative luciferase unit (RLU) in GLOMAX Luminometer (Promega, Madison, WI). The reaction was stopped using 25 µl of Stop & Glo solution, and the Renilla Luciferase (RL) unit was measured. Each assay was performed in triplicate, and each extract repeated three times. The FF-RLU value was normalized to the RL-RLU value.

Statistical Analysis

Significant deviation of the genotype frequency from Hardy–Weinberg equilibrium at each polymorphic variant was tested by Chi square with 1 degree of freedom. Haploview 4.2 (Daly Lab, Broad Institute, Cambridge, MA, USA) was used to test the LD block and predict the haplotypes for the *APOE* markers. For association study, the data were evaluated for *p*-value, odds ratio (OR), and 95% confidence interval (CI) by means of the 2×2 contingency Chi -square analysis (statpages.org/ctab 2×2.html) by John C. Pezzullo, Georgetown University, Washington, DC, USA. Paired *t* test was performed using GraphPad Prism 5. p-value < 0.05 with 95% CI or < 0.01 with 99% CI being considered significant.

Results

All the SNPs selected for this study were in Hardy–Weinberg equilibrium. The allele and genotype frequencies for *APOE* variants were compared between 302 eastern Indian PD patients and 302 ethnically matched control subjects. A significant association between PD and *APOE* ε4 polymorphism [p-value = 0.0003, OR (95% CI) = 1.95 (1.34–2.85)] was found. This association was also reflected in *APOE* ε3/ε4 genotype [p-value = 0.0001, OR (95% CI) = 2.45 (1.56–2.79)] posing a similar risk. On the other hand, *APOE* ε3 was observed to be present in significantly higher number in control compared to the PD patients both in allelic [p-value = 0.028, OR (95% CI) = 0.68 (0.49–0.94)] and genotypic (ε3/ε3) [p-value = 0.007, OR (95% CI) = 0.61 (0.42–0.88)] form (Table 1). However, no significant association was found between the *APOE* promoter SNPs and PD (Table 1). Interestingly, the haplotype (AG) constructed from these promoter SNPs, was significantly over represented in female patients (Table 2) suggesting a gender specific association [p-value = 0.037, OR (95% CI) = 1.52 (1.02–2.26)] between PD and *APOE* posing risk to female patients.

No significant association was observed ($p > 0.05$) after analyzing the allelic and the genotypic distribution of *CTSD* polymorphism among the 220 PD cases and 248 control individuals (Table 3). Since age and positive family history increase the susceptibility of patients to the disease, the patient group was subdivided into early (EOPD, age at onset < 40 years) and late (LOPD, age at onset ≥ 40 years) onset categories. On comparing the EOPD and LOPD patients as well as patients with positive and negative family histories of any neurological diseases, no significant association of PD with any specific allele or genotype of *CTSD* polymorphisms was observed (Table S2 and S3). The combined effect of *APOEε4* carrier/noncarrier with *CTSD* 'T' allele on the risk of development of PD in the same cohort was also

Table 1 Frequencies of allelic and genotypic distribution of *APOE* (coding and promoter SNPs) among PD patients and control

Polymorphisms	Allele/genotype	Patients $N=302$ (%)	Control $N=302$ (%)	OR (95% CI)	p-Value‡
Coding SNPs (rs7412 and rs429358)	ε2	20 (3.3)	30 (5)	0.66 (0.35–1.21)	0.19
	ε3	492 (81.5)	523 (86.6)	**0.68 (0.49–0.94)**	**0.028***
	ε4	92 (15.2)	54 (8.4)	**1.95 (1.34–2.85)**	**0.0003****
	ε2/ε3	16 (5.3)	26 (8.6)	0.59 (0.29–1.18)	0.15
	ε2/ε4	4 (1.3)	4 (1.3)	1.00 (0.21–4.79)	1.00
	ε3/ε3	198 (65.6)	229 (75.8)	**0.61 (0.42–0.88)**	**0.007***
	ε3/ε4	80 (26.5)	39 (13)	**2.45 (1.56–2.79)**	**0.0001****
	ε4/ε4	4 (1.3)	4 (1.3)	1.00 (0.21–4.79)	1.00
Promoter SNP (rs449647)†	A	535 (88.6)	522 (86.4)	1.22 (0.85–1.74)	0.30
	T	69 (11.4)	82 (13.6)		
	A/A	234 (77.5)	225 (74.5)	1.18 (0.80–1.74)	0.45
	A/T	67 (22.2)	72 (23.8)	0.91 (0.61–1.35)	0.70
	T/T	1 (0.3)	5 (1.7)	0.20 (0.01–1.74)	0.22
Promoter SNP (rs405509)†	T	347 (57.5)	342 (56.7)	1.03 (0.82–1.31)	0.82
	G	257 (42.5)	262 (43.3)		
	T/T	100 (33.1)	87 (28.8)	1.22 (0.85–1.76)	0.29
	T/G	147 (48.7)	168 (55.6)	0.76 (0.54–1.06)	0.10
	G/G	55 (18.2)	47 (15.6)	1.21 (0.77–1.89)	0.45

All the positive associations were marked in bold

**APOE*ε3 allele is significantly overrepresented in control than in the patients which deciphers protection. This is sustained at the genotype (*ε*3/*ε*3) level as well

***APOE*ε4 allele is significantly higher in patients than in control and acts as a risk factor. The risk is maintained at the genotype level when it is present even with ε3

†No significant association was found for the *APOE* promoter SNPs

‡*p*-value for the association of the allele or genotype with respect to patients and control

N number of subjects, *n* number of chromosomes, *OR* odds ratio, *CI* confidence interval

Table 2 Distribution of *APOE* promoter haplotypes in female and male PD patients

Promoter Haplotypes[#] rs449647rs405509	Female PD $n=144$ (%)	Male PD $n=460$ (%)	OR (95% CI)	p-Value
AT	66 (45.8)	251 (54.57)	0.71 (0.48–1.04)	0.070
AG	63 (43.75)	156 (33.9)	**1.52 (1.02–2.26)**	**0.037*****
TT	9 (6.25)	32 (6.96)	0.89 (0.39–2.01)	0.853
TG	6 (4.2)	21 (4.57)	0.91 (0.32–2.44)	1.000

All the positive associations were marked in bold

[#]Promoter haplotypes (for rs449647 and rs405509) were constructed using Haploview 4.2 software and sex-specific distribution of the haplotypes were checked

***AG haplotype was observed to be significantly overrepresented in the female PD compared to the male PD, which confers risk

evaluated (Table S4), but it did not yield any association with the disease progression in the eastern Indian patients.

To determine the role of *BDNF*, two SNPs (rs6265 and rs56164415) were analyzed among the 300 cases and 304 controls (Table 4). No significant association was observed between PD and either of the two SNPs. However, genotype analysis revealed that 'T/T' genotype of rs56164415 (C270T) was significantly overrepresented as compared to the 'C/C' genotype (Table 5) in the EOPD patients than the LOPD group [p-value = 0.02, OR (95% CI = 5.09 (1.23–22.25)]. This was further validated using luciferase assay, which confirmed a significantly lower expression of luciferase gene in the presence of 'T/T' genotype of *BDNF* promoter variant compared to 'C/C' genotype (Fig. 1).

Table 3 Frequency of allelic and genotypic distribution of *CTSD* coding SNP (rs17571) among PD patients and control

Allele/Genotype	Patients $N=220$ (%)	Control $N=248$ (%)	OR (95% CI)	p-Value†
C	397 (90.2)	454 (91.5)	0.85 (0.53–1.37)	0.50
T	43 (9.8)	42 (8.5)		
C/C	178 (80.9)	207 (83.5)	0.84 (0.51–1.39)	0.51
C/T	41 (18.6)	40 (16.1)	1.19 (0.72–1.98)	0.54
T/T	1 (0.5)	1 (0.4)	1.13 (0.03–41.47)	1.00

No significant change in distribution was observed between cases and control either at allelic or genotypic level for the coding SNP of *CTSD*

† p-value for the association of the genotype or allele with respect to patients and control

N number of subjects, *OR* odds ratio, *CI* confidence interval

Table 4 Frequencies of allelic and genotypic distribution of *BDNF* SNPs (rs56164415 and rs6265) among PD patients and control

Polymorphisms	Allele/genotype	Patients $N=300$ (%)	Control $N=304$ (%)	OR (95% CI)	p-Value
rs56164415	C	471 (78.5)	452 (74.3)	1.26 (0.96–1.66)	0.09
	T	129 (21.5)	156 (25.7)		
	C/C	181 (60.3)	170 (55.9)	1.20 (0.86–1.68)	0.28
	C/T	109 (36.4)	112 (36.8)	0.98 (0.69–1.38)	0.93
	T/T	10 (3.3)	22 (7.3)	0.44 (0.19–1.00)	0.04
rs6265	G	448 (74.7)	458 (75.3)	0.97 (0.74–1.26)	0.79
	A	152 (25.3)	150 (24.7)		
	G/G	170 (56.7)	170 (56)	1.03 (0.74–1.44)	0.87
	G/A	108 (36)	118 (38.8)	0.89 (0.63–1.25)	0.50
	A/A	22 (7.3)	16 (5.2)	1.42 (0.77–2.91)	0.32

Table 5 Frequencies of allelic and genotypic distribution of *BDNF* SNPs (rs56164415 and rs6265) with age of onset among PD patients (EOPD vs LOPD)

Polymorphisms	Allele/genotype	EOPD $N=72$ (%)	LOPD $N=228$ (%)	OR (95% CI)	p-Value
rs56164415–	C	109 (75.7)	362 (79.4)	0.81 (0.51–1.29)	0.35
	T	35 (24.3)	94 (20.6)		
	C/C	43 (59.7)	138(60.5)	1.01 (0.57–1.80)	1.00
	C/T	23(32)	86 (37.7)	0.78 (0.42–1.41)	0.40
	T/T	6 (8.3)	4 (1.8)	**5.09 (1.23–22.25)**	**0.02***
rs6265	G	107 (74.3)	341 (74.8)	0.98 (0.62–1.53)	0.91
	A	37 (25.7)	115 (25.2)		
	G/G	39 (54.2)	131 (57.5)	0.88 (0.50–1.54)	0.68
	G/A	29 (40.2)	79 (34.6)	1.27 (0.71–2.27)	0.40
	A/A	4 (5.6)	18 (7.9)	0.69 (0.19–2.26)	0.61

All the positive associations were marked in bold

*'T/T' genotype of rs56164415 is significantly over represented in EOPD than in LOPD patients

EOPD early onset Parkinson's disease (<40 years), *LOPD* late onset Parkinson's disease (≥40 years), *N* number of subjects, *n* number of chromosomes, *OR* odds ratio, *CI* confidence interval

Discussion

APOE plays a significant role in cholesterol metabolism, mitochondrial functioning, and maintenance of neuronal activities (Liu et al. 2013). This protein has two important domains—LDL receptor-binding region (N-terminal) and major lipid-binding region (C-terminal) which are connected by a "Hinge'' region. In APOEε3 isoform, Cys112 makes a salt bridge with Arg61 of the LDL receptor-binding domain. Glu255, present on major lipid-binding domain, can freely interact with its counterpart. However, in APOEε4 isoform, Cys112-Arg polymorphism prevents this salt bridge formation, thereby allowing Arg61 to

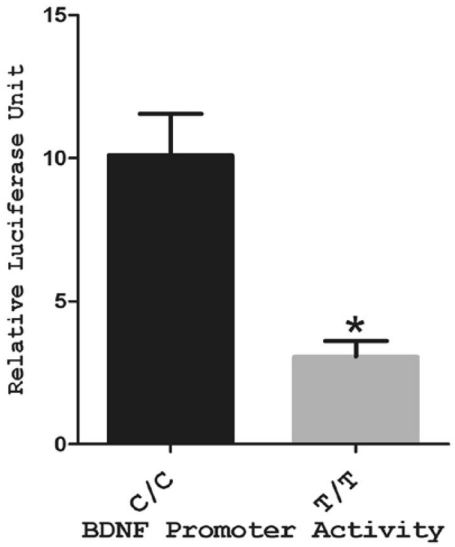

Fig. 1 Evaluation of *BDNF* Promoter Activity. Plasmid constructs harboring 'T/T' genotype of rs56164415 (C > T) showing significant reduced activity of firefly in SH-SY5Y cell line [*p*-value = 0.012]. Data are presented as relative fold change of firefly calculated as FF-RLU/RL-RLU of the sample, compiled from three independent experiments. Statistical significance was determined by paired t-test using Graph Pad Prism 5 (*$p < 0.05$). Error bars represent standard deviations (SD)

interact with Glu255. This affects the overall folding of the protein wherein it forms a molten globule (partially unfolded)-like structure which can bind to lipid particles, such as lipoproteins or lysosomal membranes, with higher affinities (Hauser et al. 2011). Along with this, the domain–domain interaction of APOEε4 also leads to mitochondrial dysfunction in neuronal cells (Chen et al. 2011). This suggests that APOE may be involved in the mitochondrial pathway associated with neurodegenerations. In PD, it has been reported that mitochondrial integrity maintenance is essential, and any insult to mitochondria may lead to the disease pathogenesis (Larsen et al. 2018). A C-terminal-truncated fragment (amino acids, 1-272) of APOEε4 can bind to the mitochondrial complexes III and IV and inhibit their activities, thereby leading to neurodegeneration (Nakamura et al. 2009). While both *ε3* allele and *ε3/ε3* genotype were found to be overrepresented among the control subjects, a significant risk for PD was observed for those patients carrying *ε4* allele or the *ε3/ε4* genotype. This suggests that the *ε3* allele possibly plays a protective role against neurodegeneration. As structurally APOEε4 is toxic for the cells, the presence of one allele is enough to generate the toxicity. Among *ε3/ε4* individuals, the presence of the protective *ε3* allele could not override the toxic effect of *ε4* as observed in our study.

On the other hand, it has been shown in a previous study that SP1 and GATA can bind with -491 (rs449647) and -219 (rs405509) polymorphic sites of *APOE* promoter and can regulate its expression (Maloney et al. 2010). An in vitro study also revealed that expression of *APOE* was regulated by estrogen (Lambert et al. 2004). APOE plays a major role in neuronal function. Recent study also shows that APOE has a neuroprotective role in aging (Hudry et al. 2019). Several epidemiological studies have reported that the incidence and prevalence of PD are higher in postmenopausal than in premenopausal women of similar age. In menopausal state, the female lacks estrogen thereby increasing the risk of Parkinson's disease (Ragonese et al. 2004; Currie et al. 2004). From our association study, we have tried to connect these two independent observations where we found that a specific haplotype containing A (-491) and G (-219) of *APOE* promoter SNPs increases the risk of PD in female patient of our cohort, and 64% of our female PD patients belong to the age beyond 40 years which is expected to include the menopausal phase. The menopausal status of the female PD patients of this cohort, however, needs to be confirmed to ratify this claim. However, this novel finding suggests that *APOE* promoter SNPs might play a key role in controlling the expression of APOE in a sex-specific manner.

The c.224 C > T polymorphism of *CTSD* in exon 2 has been reported to be associated with the increased procathepsin D expression and altered intracellular maturation (Schulte et al. 2003). The T allele of *CTSD* is also known to be associated with the increased risk of AD (Singh et al. 2014). Contrary to the previous reports, no such association was observed in the eastern Indian PD patient cohort. A previous study involving German population suggested an association between a combination of the *ε4* allele of *APOE* and T allele of *CTSD* with PD (Ntais et al. 2004); however, no such association was observed in this study.

Previous studies have examined the possible role of *BDNF* polymorphism in the pathogenesis of PD due its established role as a trophic factor for dopaminergic neurons. Decreased BDNF mRNA expressions and protein content have been observed in the substantia nigra of PD patients (Parain et al. 1999; Howells et al. 2000). Based on these observations, increasing molecular epidemiological studies have focused on the associations between *BDNF* polymorphisms and PD risk. Most commonly studied SNPs include G196A (rs6265) and C270T (rs56164415). However, the roles of these two SNPs on the susceptibility of PD have been inconsistent (Zintzaras and Hadjigeorgiou 2005; Saarela et al. 2006; Karakasis et al. 2011; Lee and Song 2014). No significant association has been identified for G196A (rs6265) in this cohort, which is consistent with the previous findings (Dai et al. 2013; Mariga et al. 2017).

Reduced expression of BDNF also has been observed in the postmortem brain (Parain et al. 1999). BDNF plays many crucial roles including neurogenesis, synapse formation, axonal guidance, and neuronal differentiation and the

loss of BDNF contributes to reduced expression of synaptic proteins, which may lead to the loss of synaptic plasticity (Howells et al. 2000). The 'T/T' genotype in the EOPD patients was found to be more predominant than the 'C/C' genotype, and the promoter activity assay revealed that the expression of luciferase gene in the presence of 'T/T' genotype of *BDNF* promoter variants (rs56164415) was reduced significantly compared to the 'C/C' genotype. Therefore, a decreased BDNF expression might contribute to the pathogenesis of PD in these patients, although further studies are required to confirm the BDNF protein-level expression in these patients.

In conclusion, this is the first study investigating the roles of *APOE*, *CTSD*, and *BDNF* polymorphisms in PD pathogenesis among the eastern Indian population. While *APOE ε4* allele puts patients at a risk of developing PD, the *ε3* allele appears to be imparting protection in this cohort. In addition, an *APOE* promoter haplotype (AG) has been identified in female PD patients causing risk. However, there was no significant association between *CTSD* and PD in this cohort. The 'T/T' genotype of *BDNF* promoter variant (rs56164415) was found to be a risk factor for the eastern Indian EOPD patients. Promoter activity results further confirmed the reduced expression of BDNF in the presence of 'T/T' genotype, which possibly explains the susceptibility of patients to EOPD.

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Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflicts of interest to disclose.

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